

Exhibit A

U.S. Patent No. 7,598,365



US007598365B2

(12) **United States Patent**
D'Halluin et al.(10) **Patent No.:** **US 7,598,365 B2**
(45) **Date of Patent:** **Oct. 6, 2009**(54) **TARGETED DNA INSERTION IN PLANTS**

WO WO 03/080809 A2 10/2003

(75) Inventors: **Kathleen D'Halluin**, Mariakerke (BE);
Chantal Vanderstraeten, Ghent (BE);
Rene Ruiter, Heusden (BE)(73) Assignee: **Bayer Bioscience N.V.** (BE)(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 232 days.(21) Appl. No.: **10/580,076**(22) PCT Filed: **Nov. 17, 2004**(86) PCT No.: **PCT/EP2004/013122**§ 371 (c)(1),
(2), (4) Date: **May 18, 2006**(87) PCT Pub. No.: **WO2005/049842**PCT Pub. Date: **Jun. 2, 2005**(65) **Prior Publication Data**

US 2006/0282914 A1 Dec. 14, 2006

(30) **Foreign Application Priority Data**

Nov. 18, 2003 (EP) 03078700

(51) **Int. Cl.****C07H 21/04** (2006.01)
C12N 15/82 (2006.01)
C07K 14/00 (2006.01)
A01H 5/00 (2006.01)(52) **U.S. Cl.** **536/23.2**; 536/23.4; 800/278;
800/320.1; 435/468; 435/419; 530/350(58) **Field of Classification Search** None
See application file for complete search history.(56) **References Cited****U.S. PATENT DOCUMENTS**5,474,896 A * 12/1995 Dujon et al. 435/6
5,689,052 A 11/1997 Brown et al.
2002/0107214 A1 * 8/2002 Chouluka et al. 514/44**FOREIGN PATENT DOCUMENTS**CA 2 451 492 1/2003
EP 0 242 236 A1 10/1987
EP 0 242 246 A1 10/1987
EP 0 317 509 A2 5/1989
WO WO 94/01560 A1 1/1994
WO WO 94/17176 A1 8/1994
WO WO 94/18313 A1 8/1994
WO WO 94/26913 11/1994
WO WO 95/09233 A1 4/1995
WO WO 96/14408 A2 5/1996
WO WO 00/46386 A2 8/2000
WO WO 03/004659 A2 1/2003
WO WO 03/054189 A2 7/2003**OTHER PUBLICATIONS**Guo et al., (2004) Protein Tolerance To Random Amino Acid Change
P.N.A.S. 101 (25) 9205-9210.*
Lazar et al., (1988) Transforming Growth Factor A: Mutation Of
Aspartic Acid 47 And Leucine 48 In Different Biological Activities.
Molec. & Cell. Biol. 8(3)1247-52.*
Dujon, et al., 95.6% identicals to SEQ ID No. 1, found in the Issued
Patents Database. Sequence No. 2 from 5474896-A US, issued on
Dec. 12, 1995.*
Chilton et al. Targeted Integration of T-DNA into the Tobacco
Genome at Double-stranded Breaks: New Insights on the
MEchanism of T-DNA integration. (2003) Plant Physiology ; vol.
133, pp. 956-965.*
Raikhel, N. Nuclear Targeting in Plants. (1992) Plant Physiology;
vol. 100; pp. 1627-1632.*
Ashby, et al. "Ti Plasmid-Specified Chemotaxis of *Agrobacterium*
tumefaciens C58C¹ toward vir-Inducing Phenolic Compounds and
Soluble Factors from Monocotyledonous and Dicotyledonous
Plants", Journal of Bacteriology, vol. 170, No. 9, Sep. 1988, p. 4181-
4187.
Bolton, et al., "Plant Phenolic Compounds Induce Expression of the
Agrobacterium tumefaciens Loci Need for Virulence", Science, vol.
232, p. 983-985, 1986.
Chalfie, et al., "Green Fluorescent Protein as a Marker for Gene
Expression", Science, vol. 263, p. 802-805, Feb. 11, 1994.
Chilton and Que, "Targeted Integration of T-DNA into the Tobacco
Genome at Double-Stranded Breaks: New Insights on the Mecha-
nism of T-DNA Integration", Plant Physiology, vol. 133, p. 956-965,
Nov. 2003.
Chouluka et al., "Induction of Homologous Recombination in Mam-
malian Chromosomes by Using the I-SceI System of *Saccharomyces*
cerevisiae", Molecular and Cellular Biology, vol. 15, No. 4, p. 1968-
1973, Apr. 1995.
Colleaux, et al., "Recognition and Cleavage Site of the Intron-En-
coded *omega* Transposase", Proc. Natl. Acad. Sci. USA, vol. 85, p.
6022-6026, Aug. 1988.
Cramer, et al., "Improved Green Fluorescent Protein by Molecular
Evolution Using DNA Shuffling", Nature Biotechnology, vol. 14, p.
315-319, Mar. 1996.
De Block, et al., "Engineering Herbicide Resistance in Plants by
Expression of a Detoxifying Enzyme", The EMBO Journal, vol. 6,
No. 9, p. 2513-2518, 1987.
Fennoy, et al., "Synonymous Codon Usage in *Zea mays* L. Nuclear
genes is Varied by levels of C and G-ending Codons", Nucleic Acids
Research, vol. 21, No. 23, p. 5294-5300, 1993.
Guivarc'h, et al., "Localization of Target Cells and Improvement of
Agrobacterium-mediated Transformation Efficiency by Direct
Acetosyringone Pretreatment of Carrot Root Discs", Protoplasma,
vol. 174, p. 10-18, 1993.
Isalan, et al., "A Rapid, Generally Applicable Method to Engineer
Zinc Fingers Illustrated by Targeting the HIV-1 Promoter", Nature
Biotechnology, vol. 19, p. 656-660, Jul. 2001.

(Continued)

Primary Examiner—Cathy Kingdon Worley
(74) *Attorney, Agent, or Firm*—Hunton & Williams LLP(57) **ABSTRACT**Methods and means are provided to improve targeted DNA
insertion in plants using rare-cleaving "double stranded
break" inducing enzymes. Also provided are improved I-SceI
encoding nucleotide sequences.**20 Claims, 1 Drawing Sheet**